Journal of Medicinal Chemistry

Volume 44, Number 10 May 10, 2001

Letters

Highly Selective and Potent Neuropeptide Y (NPY) Y1 Receptor Antagonists Based on [Pro^{30}, Tyr^{32}, Leu^{34}]NPY(28-36)-NH$_2$ (BW1911U90)†

Ambikaipakan Balasubramaniam,*,‡ Vikas C. Dhawan,‡ Deborra E. Mullins,§ William T. Chance,‡,|| Sulaiman Sheriff,‡ Mario Guzzi,§ M. Prabhakaran,‡ and Eric M. Parker§

Division of Gastrointestinal Hormones, Department of Surgery and Interdisciplinary Neurosciences Program, University of Cincinnati and VA Medical Centers, Cincinnati, Ohio 45267-0558, and Department of Central Nervous System and Cardiovascular Research, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033-0539

Received January 23, 2001

Neuropeptide Y (NPY), a 36-residue peptide amide isolated originally from porcine brain, is a member of a family of homologous hormones including peptide YY (PYY) and pancreatic polypeptide (PP).[1] It is the most abundant peptide present in the mammalian brain and has been shown to exhibit a wide spectrum of central and peripheral activities. These actions of NPY are mediated by at least six G-protein coupled receptor subtypes denoted as Y1, Y2, Y3, Y4, Y5, and y6 (see ref 2 for a review). All these receptors are coupled to signal transduction pathways regulating levels of both intracellular calcium and cAMP.

Investigations to date have implicated NPY in the pathophysiology of a number of diseases including feeding disorders, seizures, anxiety, hypertension, and diabetes.[2,3] To aid in the determination of the NPY receptor subtype(s) responsible for the physiological and pathophysiological effects of NPY, efforts have been made to develop highly potent and selective ligands for various NPY receptors.[2,3] Since the Y1 receptor was one of the first to be characterized and because of its involvement in the regulation of appetite and blood pressure,[4−6] a wide variety of peptide and non-peptide Y1 receptor antagonists have been developed to date. These ligands, including compounds **1** and **6** (Table 1), T-190, T-241, BIBP3226, BIBO 3304, SR120819A, J-104870, and compounds based on 1,2,3-trisubstituted indole and 1,3-disubstituted benzazepines, have proven useful in delineating Y1 mediated activity.[2−4,7−14] However, problems associated with solubility, stability, toxicity, and/or selectivity may limit their in vivo and/or clinical utility. One of these compounds, **6**, developed by Daniels and co-workers,[8] exhibited subnanomolar affinity to Y1 receptors and has therefore found widespread applicability in defining the role and distribution of Y1 receptors.[15−17] However, our recent investigations revealed that **6** and its parent compound, **1**, as well as our Y1 antagonists, T-190 and T-241, exhibit potent agonist activity at the Y4 receptors.[7] This paper describes structure−activity studies with compound **1**, undertaken toward dissociating the Y1 and Y4 activities, that have resulted in the development of a series of potent Y1 antagonists that exhibit little or no activity at the Y2, Y4, and Y5 receptors.

Table 1. C-Terminal Nonapeptide Monomer and Dimer Analogues of NPY

#	Structures
1 (BW1911U90)	Ile–Asn-Pro-Ile-Tyr-Arg-Leu-Arg-Tyr-NH_2
2	Ile–Asn-Pro-Ile-Tyr-Arg-Leu-Arg-Tyr-OMe
3	Ile–Asn-Pro-Ile-Tyr-Arg-Leu-Arg-(CH_2-NH)-Tyr-NH_2
4	Ile–Asn-Pro-Cys-Tyr-Arg-Leu-Arg-Tyr-OMe | Ile–Asn-Pro-Cys-Tyr-Arg-Leu-Arg-Tyr-OMe
5	Ile–Asn-Pro-Cys-Tyr-Arg-Leu-Arg-(CH_2-NH)-Tyr-NH_2 | Ile–Asn-Pro-Cys-Tyr-Arg-Leu-Arg-(CH_2-NH)-Tyr-NH_2
6 (GR231118)	Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-NH_2 Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-NH_2
7	Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-OMe Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-OMe
8	Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-(CH_2-NH)-Tyr-NH_2 Ile–Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-(CH_2-NH)-Tyr-NH_2

† Supported by NIH Grants GM47122 and DK53548 and a VA Merit Review Grant.

* Address correspondence to A. Balasubramaniam. Tel: (513)558-3819. Fax: (513)558-0750. E-mail: Ambi.bala@UC.EDU.
‡ University of Cincinnati College of Medicine.
§ Schering-Plough Research Institute.
|| VA Medical Center.

10.1021/jm010031k CCC: $20.00
Published on Web 04/18/2001

Table 2. Affinities of C-Terminal Nonapeptide Monomer and Dimer Analogues of NPY for Cloned "Y"-Receptors (K_i, nM)[a]

peptide	K_i (nM) Y1	Y2	Y4	Y5	selectivity Y1/Y2/Y4/Y5
NPY	0.52 ± 0.02	0.23 ± 0.02	0.08 ± 0.003[b]	2.21 ± 0.03	
1[c]	5.0 ± 0.46	11.3 ± 3.8	5.8 ± 0.91	>1000	1/2/1/200
2	25.7 ± 5.9	1420 ± 191	2403 ± 368	7100 ± 380	1/50/90/280
3	34.8 ± 5.8	1650 ± 114	3737 ± 182	5080 ± 940	1/50/110/150
4	4.8 ± 1.1	1120 ± 15	99 ± 3	464 ± 17	1/230/20/100
5	2.3 ± 0.5	822 ± 78	65 ± 5	2340 ± 370	1/360/30/1020
6[c]	0.07 ± 0.01	55 ± 21	0.26 ± 0.08	115 ± 29	1/790/3/1640
7	0.27 ± 0.01	1036 ± 116	33.4 ± 1.7	662 ± 49	1/3840/120/2450
8	0.46 ± 0.12	624 ± 69	65.5 ± 1.9	7890 ± 243	1/1360/140/17150

[a] Affinities for Y1, Y2, and Y5 receptors were determined using ^{125}I-PYY, while those for Y4 receptors were determined using ^{125}I-PP. [b] PP was used as control in cells expressing Y4 receptors. [c] Data are from ref 7.

Results and Discussion. All the peptides used in this study were >96% homogeneous by reversed-phase analytical chromatography in two diverse solvent systems, and they had the expected mass and amino acid composition (see Supporting Information). Compound **6** and its analogues, **7** and **8**, were synthesized by solid-phase dimerization of the partially protected monomers.[14] In our hands, this strategy was found to be superior to the solution dimerization technique reported by Angus and co-workers[18] for the synthesis of **6**.

Compound **6** has proven to be an indispensable tool in NPY research.[7,11−13] However, recent findings that compound **6** also exhibits potent agonist activity at the Y4 receptors[3,7] cast doubt on the studies that have used this compound to investigate the role and distribution of Y1 receptors. To alleviate this problem, we initiated a systematic investigation with the related peptide **1** to develop analogues devoid of Y4 activity. Moreover, we postulated that this goal could be accomplished via modifications in the C-terminus region because this end of NPY has been shown to be crucial for triggering biological activity. Accordingly, replacement of the C-terminal amide in **1** with methyl ester as in compound **2** imparted greater Y1 selectivity relative not only to Y4 but also to Y2 and Y5 compared to **1** (Tables 1 and 2). Similarly, replacement of the peptide bond between Arg^{35} and Tyr^{36} with Ψ(CH$_2$-NH) as in compound **3** induced selectivity for Y1 receptors comparable to that of methyl ester modification in compound **2**. Therefore, it was of great interest to synthesize a compound having both Ψ(CH$_2$-NH)$^{35-36}$ and C-terminal methyl ester modifications to determine whether this will have an additive effect on Y1 selectivity. Unfortunately, our attempts to synthesize such a compound by the solid-phase method proved futile because of the apparent resistance of the [Pro^{30}, Tyr^{32}, Leu^{34}, Ψ(CH$_2$-NH)$^{35-36}$]-NPY(28-36)-O-Merrifield resin to transesterfication. The loss of electron-withdrawing ability of the C-terminal peptide bond between Arg^{35}-Tyr^{36} due to −CO−NH− → −CH$_2$−NH− modification may be responsible for this inertness. At present, we are pursuing other strategies including solution-phase methods to synthesize this compound.

Despite their increased selectivity for Y1 receptors, both **2** and **3** exhibited 5−7 times lower affinity for this receptor than the parent compound **1** (Table 2). Since NMR studies have revealed that NPY exists as a dimer in solution[19] and because Daniels and co-workers[10] and our laboratory[8] have shown that dimerization increases Y1 affinity, we synthesized compounds **4** and **5**, the Cys^{31} dimers of compounds **2** and **3**, respectively (Table 1). Dimerization restored the Y1 affinity (Table 2).

Table 3. Agonist (EC_{50}, nM) and Antagonist (K_b, nM) Potencies of the C-Terminal Nonapeptide Dimer Analogues of NPY for the Inhibition of Forskolin Stimulated cAMP Synthesis in Cells Expressing Cloned "Y"-Receptors

peptide	EC_{50} or K_b (nM) Y1[a]	Y2	Y4	Y5
NPY	0.52 ± 0.04	0.46 ± 0.04	0.16 ± 0.08[b]	7.89 ± 1.29
4	**2.30 ± 0.58**	no effect[c]	no effect	no effect
5	**0.79 ± 0.09**	no effect	no effect	no effect
6[d]	**0.04 ± 0.02**	983 ± 217	2.5 ± 0.54	2050
7	**0.19 ± 0.01**	no effect	no effect	no effect
8	**0.58 ± 0.10**	no effect	no effect	no effect

[a] K_b values are bold. [b] PP was used as control in cells expressing Y4 receptors. [c] No agonist activity at <20 000 nM. [d] Data are from ref 7. Compounds **4**, **5**, **7**, and **8** also did not exhibit any Y4 antagonistic activities at 100 nM (see Supporting Information).

Moreover, these analogues retained sufficient selectivity for Y1 receptors (see functional studies below).

Since cross-linked dimers such as **6** exhibited higher Y1 receptor affinity than the corresponding Cys^{31} dimer, Bis(31/31′){[Cys^{31}, Pro^{30}, Tyr^{32}, Leu^{34}]NPY(28-36)-NH$_2$},[14] we also synthesized analogues of compound **6** with C-terminal methyl ester (**7**) and Ψ(CH$_2$-NH)$^{35-36}$ (**8**), respectively (Table 1). Compounds **7** and **8** retained subnanomolar affinity for Y1 receptors and exhibited high selectivity for Y1 receptors relative to Y2, Y4, and Y5 receptors (Table 2). This is in contrast to compound **6**, which exhibited subnanomolar affinity for Y4 receptors.

To determine whether the observed Y1 selectivity based on receptor affinities also extended to functional activities, we investigated the effects of the potent analogues in this series, **4**, **5**, **7**, and **8**, on forskolin stimulated cAMP synthesis in cells expressing the Y1, Y2, Y4, or Y5 receptors (Table 3). As in the case of compounds **1** and **6**,[7] none of these analogues inhibited cAMP synthesis in Y1 cells, but they exhibited competitive antagonism of NPY. The latter was evident from the rightward shift of the inhibitory dose−response curve of NPY on cAMP synthesis in Y1 cells by the presence of any one of these peptides (100 nM) (Figure 1). The potencies were in the order of **7** > **8** > **5** > **4** (Table 3).

Compounds **4**, **5**, **7**, and **8** did not inhibit cAMP synthesis in cells expressing Y2, Y4, or Y5 receptors at concentrations <20 000 nM (Table 3; see Supporting Information also). This observation is in agreement with the lower affinities exhibited by these peptides for Y2 and Y5 receptors. On the other hand, these peptides had reasonable affinities for Y4 receptors (Table 2). Therefore, it appeared that they might behave as Y4 receptor antagonists. However, none of these peptides exhibited significant antagonistic activity in Y4 cells at a concentration of 100 nM (see Supporting Information). These

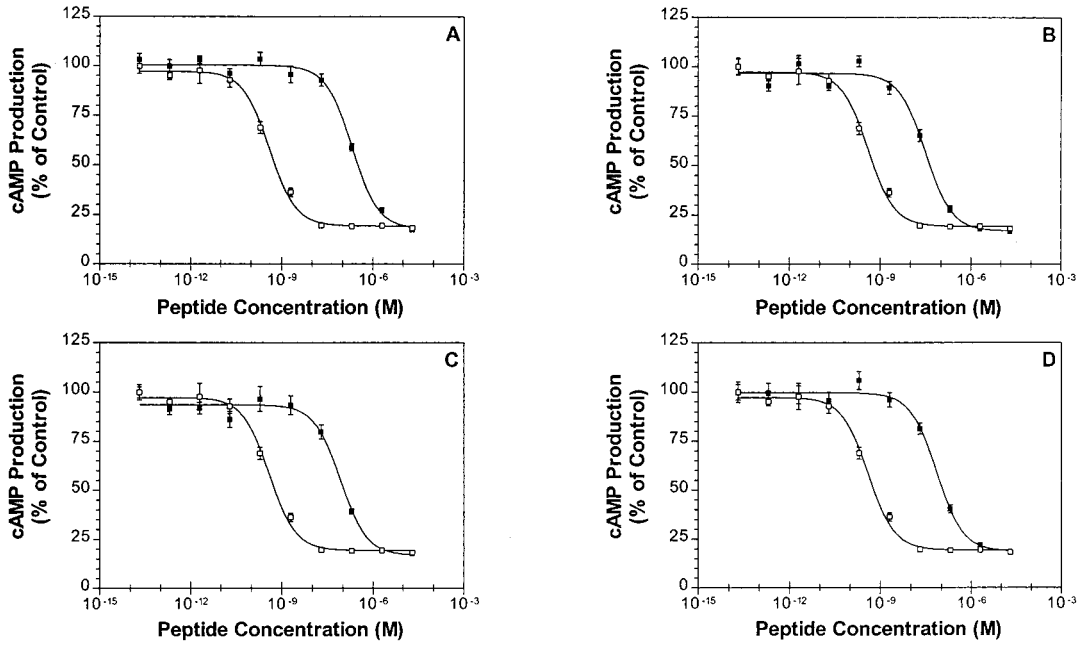

Figure 1. Compounds **4**, **5**, **7**, and **8** are competitive antagonists at the NPY Y1 receptors. Dose−response curves of NPY inhibition of forskolin stimulated cAMP synthesis in HEK 293 cells expressing the human Y1 receptors in the absence (open square) or presence of 100 nM (filled square) **7** (A), **4** (B), **5** (C), or **8** (D). See Supporting Information for experimental details.

studies confirm that compounds **4**, **5**, **7**, and **8** are potent and selective Y1 receptor competitive antagonists and suggest they could be used to detect or attenuate Y1 mediated activity without any undue concerns about interactions with other NPY receptor subtypes, especially in in vivo models.

NPY effects on feeding are mediated by both Y1 and Y5 receptors.[4−6,13] We therefore chose compound **7**, the most potent Y1 antagonist in this series, to investigate these effects. As shown in Figure 2A, pretreatment with intrahypothalamic (iht) injection of compound **7** (8.4 nmol) significantly attenuated the food intake induced by iht-NPY (0.17 nmol) during the first hour in satiated rats. On the other hand, iht injection of compound **7** (8.4 or 12.6 nmol) significantly attenuated food intake in schedule-fed rats across 1, 2, and 4 h after the injection (Figure 2B). It appears, therefore, that the effect of the specific Y1 receptor antagonist is more pronounced in fasted rats than in iht-NPY-treated satiated rats. The latter observation is in agreement with previous findings that (1) fasting, but not NPY-induced feeding, is severely affected in Y1 knockout mice[5] and (2) hypothalamic Y1 and not Y5 receptor gene expression is elevated in fasted rats.[20] Taken together, these findings suggest that the hunger signal may be predominantly mediated by NPY Y1 receptors. This inference is also supported by the fact that food intake induced by NPY, but not fasting, is significantly attenuated in Y5 knockout mice.[6]

Recent investigations have implicated NPY as an endogenous anticonvulsant agent via interaction with Y2 and/or Y5 receptors.[21] Moreover, we have often observed that iht injection of Y5 antagonists (>30 nmol) induces seizures. However, no such adverse effects are apparent with Y1 antagonists such as compound **7**. These observations tempt us to speculate that feeding disorders may be better controlled with Y1, rather than Y5, receptor selective ligands.

At present we do not know the structural features in these peptides that contribute to the high Y1 receptor selectivity. However, it is highly plausible that the

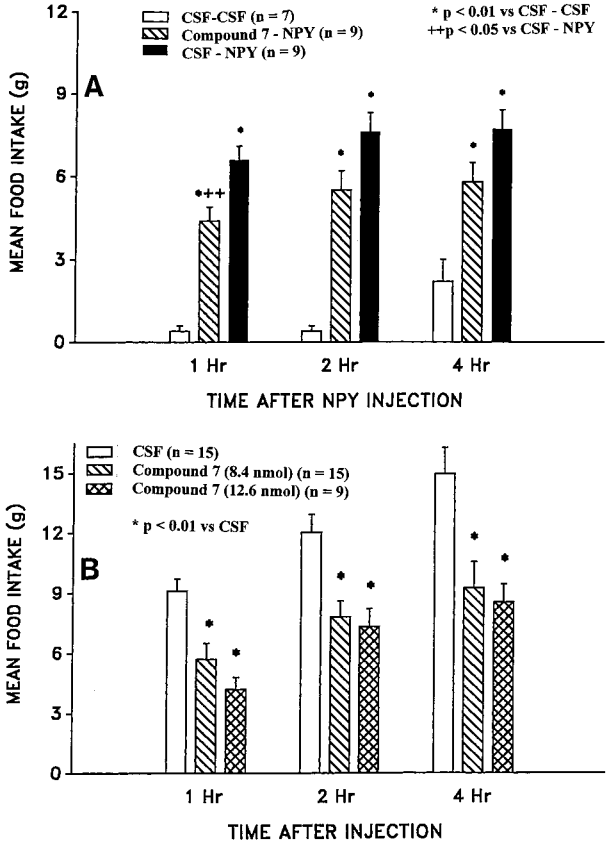

Figure 2. Compound **7** antagonized food intake in both NPY-treated and schedule-fed rats. (A) Intake of rat chow by satiated rats following the intrahypothalamic (iht) injection of NPY (0.17 nmol) preceded 10 min earlier by iht injection of compound **7** (8.4 nmol) or 1 μL of CSF. (B) Intake of rat chow by schedule-fed rats following iht injection of compound **7** (8.4 or 12.6 nmol). Data shown are the mean ± SEM. See Supporting Information for experimental details.

conformational changes induced by the loss of hydrogen bonding ability associated with the C-terminal modifications, $-CO-NH_2 \rightarrow -COOMe$ or $-CO-NH^{35-36} \rightarrow -CH_2-NH$,[35-36] may be responsible for the observed selectivity. In this regard, previous NMR studies with an analogue of compound **1**, [Tyr[32], Leu[34]]NPY(27-36)-NH_2, showed that the aromatic rings of Tyr[32] and Tyr[36] are closely associated and that the C-terminal amide is incorporated into a reverse turn via a hydrogen bond to the carbonyl oxygen of Leu[34].[9] It is possible that this hydrogen bond may play a role in bringing the Tyr rings together. The inability to form such hydrogen bonding in the methly ester analogues, **2**, **4**, and **7**, may prevent or perturb the C-terminus reverse turn, which may contribute, at least in part, to the increased Y1 selectivity. On the other hand, either a similar loss in hydrogen bonding ability and/or increased flexibility of the CH_2-NH^{35-36} in compounds **3**, **5**, and **8** may perturb the reverse turn involving C-terminal amide and contribute to the Y1 selectivity. Moreover, if our speculations are correct, then introduction of a reduced peptide bond, CH_2-NH, between Leu[34] and Arg[35] in either **1** or **6** should also result in Y1 selective analogues because of the inability to form a hydrogen bond between the carbonyl oxygen of Leu[34] and the C-terminal amide protons. These possibilities remain to be investigated.

It is intriguing that both compounds **1** and **6** behave as antagonists at Y1 receptors and agonists at Y4 receptors.[7] Although we do not know the reasons for this property, this observation is very similar to that of Hirschman and co-workers with somatostatin analogues.[22] They found somatostatin receptor agonists to be NK-1 receptor antagonists. Therefore, we could extend the same postulate developed based on a two-state receptor activation model to explain these observations.[22,23] It is possible that the binding of **1** and **6** causes productive conformational changes at Y4 but not at Y1 receptors. Hence, only the Y4 receptors become activated. Alternatively, the resting state of the Y4 receptors may be closer to the activated state and, therefore, are more easily activated than Y1 receptors, which may have to overcome a large energy barrier to become activated. Therefore, ligands may have to induce only minor changes in the Y4 receptor conformation to elicit agonist activities, while substantial changes may be required to exhibit antagonism. The reverse situation would apply for Y1 receptors.

In summary, a systematic investigation starting with compound **1** has resulted in the development of a series of highly selective and potent Y1 receptor antagonists. These compounds should prove useful in elucidating Y1 mediated activity.

Acknowledgment. We thank Dr. James Knittel for critically reading the manuscript and Dr. Koka Jayasimhulu, Director, University of Cincinnati Mass Spectral Facility, for performing MS analysis. Purchase of MS Instruments was supported in part by NIH Grant 1 S10 RR13797.

Supporting Information Available: Experimental procedures, analytical data of compounds listed in Table 1, and figures showing the lack of effects of compounds **4**, **5**, **7**, and **8** on cAMP synthesis in cells expressing NPY Y2, Y4, or Y5 receptors. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Tatemoto, K. Neuropeptide Y: Complete amino acid sequence of the brain peptide. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5485−5489.
(2) Balasubramaniam, A. Neuropeptide Y family of hormones: Receptor subtypes and antagonists. *Peptides* **1997**, *18*, 445−457.
(3) Gehlert, D. R. Multiple receptors for the pancreatic polypeptide (PP-fold) family: Physiological implications. *Proc. Soc. Exp. Biol. Med.* **1998**, *218*, 7−22.
(4) Inui, A. Neuropeptide Y feeding receptors: Are multiple subtypes involved? *Trends Pharmacol. Sci.* **1999**, *20*, 43−46.
(5) Pedrazzini, T.; Seydoux, J.; Konstner, P.; Aubert, J. F.; Grouzmann, E.; Beermann, F.; Brunner, H. R. Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. *Nat. Med.* **1998**, *4*, 722−726.
(6) Marsh, D. J.; Hollopeter, C.; Kater, K. E.; Palmiter, R. D. Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nat. Med.* **1998**, *4*, 718−722.
(7) Parker, E. M.; Babji, C. K.; Balasubramaniam, A.; Burrier, R. E.; Guzzi, M.; Hamud, F.; Mukhopadhyay, G.; Rudinski, M. S.; Tao, Z.; Tice, M.; Xia, L.; Mullins, D. E.; Salisbury, B. G. GR231118 (1229U91) and other analogues of the C-terminus of neuropeptide Y are potent neuropeptide Y1 receptor antagonists and neuropeptide Y4 receptor agonists. *Eur. J. Pharmacol.* **1998**, *349*, 97−105.
(8) Balasubramaniam, A.; Zhai, W.; Sheriff, S.; Tao, Z.; Chance, W. T.; Fischer, J. E.; Eden, P. E.; Taylor, J. Bis(31/31′){[Cys[31],Trp[32], Nva[34]]NPY(31-36)}: A Specific NPY Y-1 receptor antagonist. *J. Med. Chem.* **1996**, *39*, 811−813.
(9) Leban, J. J.; Heyer, D.; Landavazo, A.; Matthews, J.; Aulabaugh, A.; Daniels, A. J. Novel modified carboxy terminal fragments of neuropeptide Y with high affinity for Y2-type receptors and potent functional antagonism at a Y1-type receptor. *J. Med. Chem.* **1995**, *38*, 1150−1157.
(10) Daniels, A. J.; Matthews, J.; Slepetis, R. J.; Jansen, M.; Viveros, O. H.; Tadapalli, A.; Harrington, W.; Heyer, D.; Landavazo, A.; Laban, J. J.; Spaltenstein, A. High-affinity neuropeptide Y receptor antagonists. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9067−9071.
(11) Hipskind, P. A.; Lobb, K. L.; Nixon J. A.; et al. Potent and selective 1,2,3 trisubstituted indole NPY Y-1 antagonists. *J. Med. Chem.* **1997**, *40*, 3712−3714.
(12) Murakami, Y.; Hara, H.; Okada, T.; et al. 1,3-Disubstituted benzazepines as novel, potent selective neuropeptide Y Y1 receptor antagonists. *J. Med. Chem.* **1999**, *42*, 2621−2632.
(13) Kanatani, A.; Kanno, T.; Ishihara, A.; Hata, M.; Sakuraba, A.; Tanaka, T.; Tsuchiya, Y.; Mase, T.; Fukuroda, T.; Fukami, T.; Ihara, M. The novel neuropeptide Y Y1 receptor antagonist J-104870: A potent feeding suppressant with oral viability. *Biochem. Biophys. Res. Commun.* **1999**, *266*, 88−91.
(14) Daniels, A. J.; Heyer, D.; Landvazo, O.; Leban, J. K.; Spaltenstein, A. Neuropeptide Y antagonists. 1994, Patent Number WO94/00486.
(15) Kanatani, A.; Ishihara, A.; Asahi, S.; Tanaka, T.; Ozaki, S.; Ihara, M. Potent neuropeptide Y Y1 receptor anatagonist 1229U91: Blockade of neuropeptide Y-induced and physiological food intake. *Endocrinology* **1996**, *137*, 3177−3182.
(16) Ishihara, A.; Tanaka, T.; Kanatani, A.; Fukami, T.; Ihara, M.; Fukroda, T. A potent neuropeptide Y antagonist, 1229U91, suppressed spontaneous food intake in Zucker fatty rats. *Am. J. Physiol.* **1996**, *274*, R1500−R1504.
(17) Dumont, Y.; Quirion, R. [(125)I]-GR231118: A high affinity radioligand to investigate neuropeptide Y Y(1) and Y(4) receptors. *Br. J. Pharmacol.* **2000**, *129*, 37−46.
(18) Lew, M. J.; Murphy, R.; Angus, A. J. Synthesis and characterization of a selective peptide antagonist of neuropeptide Y vascular postsynaptic receptors. *Br. J. Pharmacol.* **1996**, *117*, 1768−1772.
(19) Cowely, D. J.; Hoflack, J. M.; Pelton, J. T. Structure of neuropeptide Y in solution. *Eur. J. Biochem.* **1992**, *205*, 1099−1106.
(20) Xu, B.; Kalra P. S.; Farmerie W. G.; Kalra, S. P. Endogenous neuropeptide Y (NPY) hypersecretion upregulates hypothalamic Y1 and not Y5 receptor gene expression. A role in appetite stimulation. *Neurosci. Abstr.* **1997**, *23*, 528.2.
(21) Vezzani, A.; Sperk, G.; Colmers, W. F. Neuropeptide Y: Emerging evidence for a functional role in seizure modulation. *Trends Neurosci.* **1999**, *22*, 25−30.
(22) Hirschmann, R.; Yao, W.; Cascieri, M. A.; Strader, C. D.; Maechler, L.; Cichy-Knight, M. A.; Hynes, J.; van Rijn, R. D.; Sprengeler, P. A.; Smith, A. B. Synthesis of potent cyclic hexapeptide NK-1 antagonists. Use of minilibrary in transforming a peptidal somatostatin receptor ligand into an NK-1 receptor ligand via a polyvalent peptidomimetic. *J. Med. Chem.* **1996**, *39*, 2441−2448.
(23) Leff, P. The two-state model of receptor activation. *Trends Pharmacol. Sci.* **1995**, *16*, 89−97.

JM010031K